

Porphyrins:

Chemistry and Biology

20.109 Lecture 6
24 February, 2011

Goals

- Explore some essential roles of heme in biology
- Appreciate how Nature has used the same cofactor to achieve diverse functions
- Gain some basic insight into how the cofactor properties can be tuned by its macromolecular environment

A sampling of porphyrins in Nature



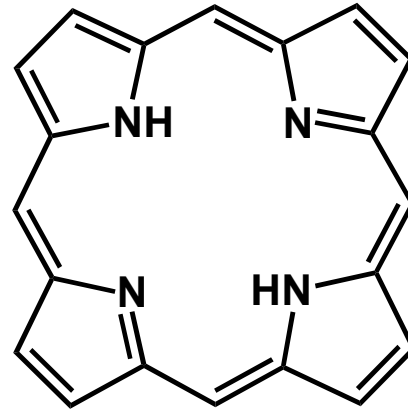
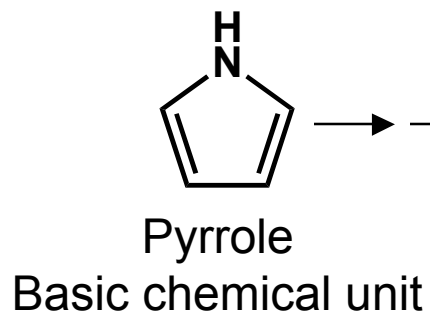
Chlorophyll



Hemoglobin

Porphyrin structure

Porphyrins are “tetrapyrroles”



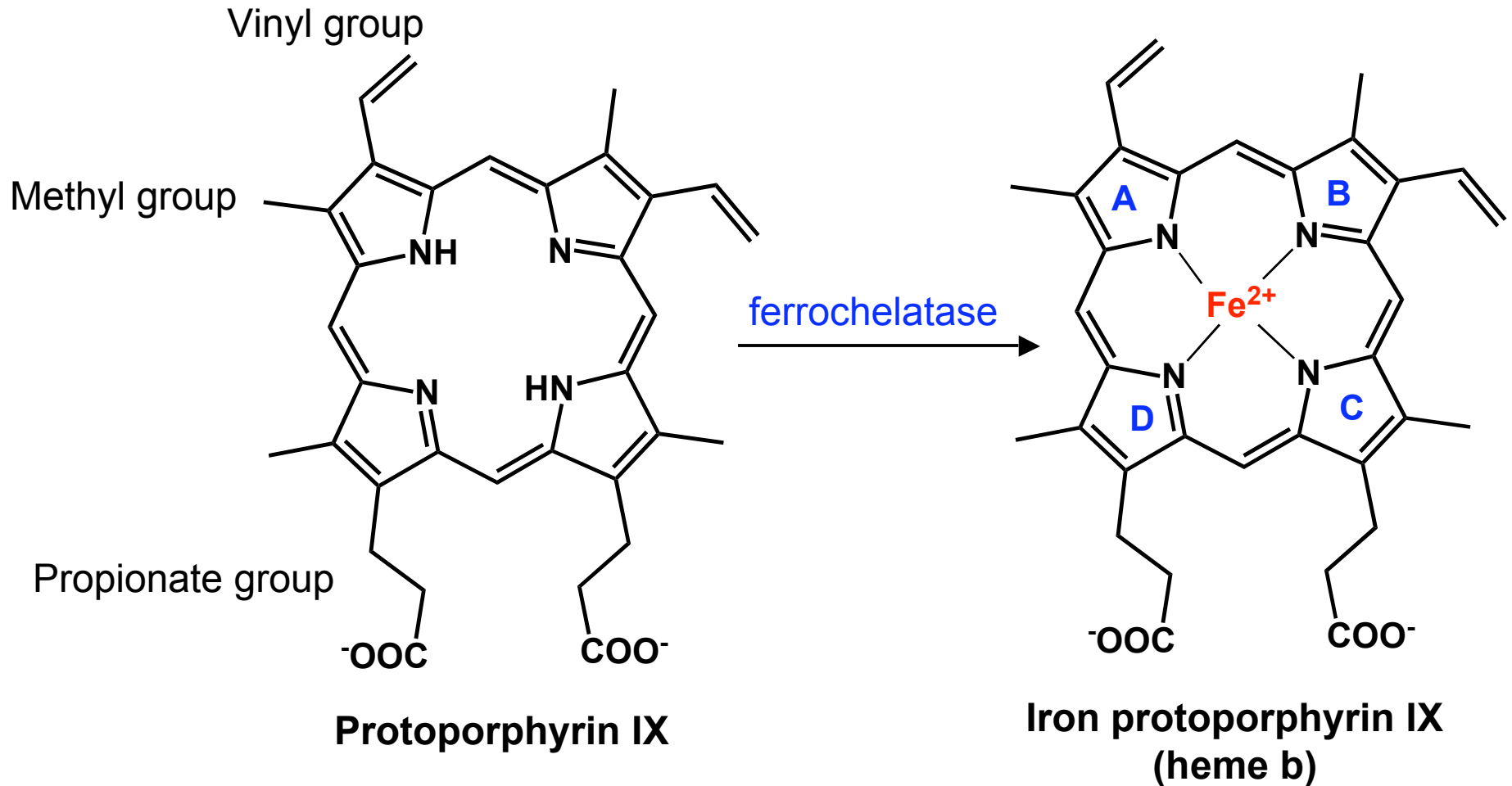
Porphine
= simplest porphyrin

Features distinguishing
porphyrins

1. Functional groups elaborated from this basic tetrapyrrole structure;
2. Identity of the coordinated metal ion

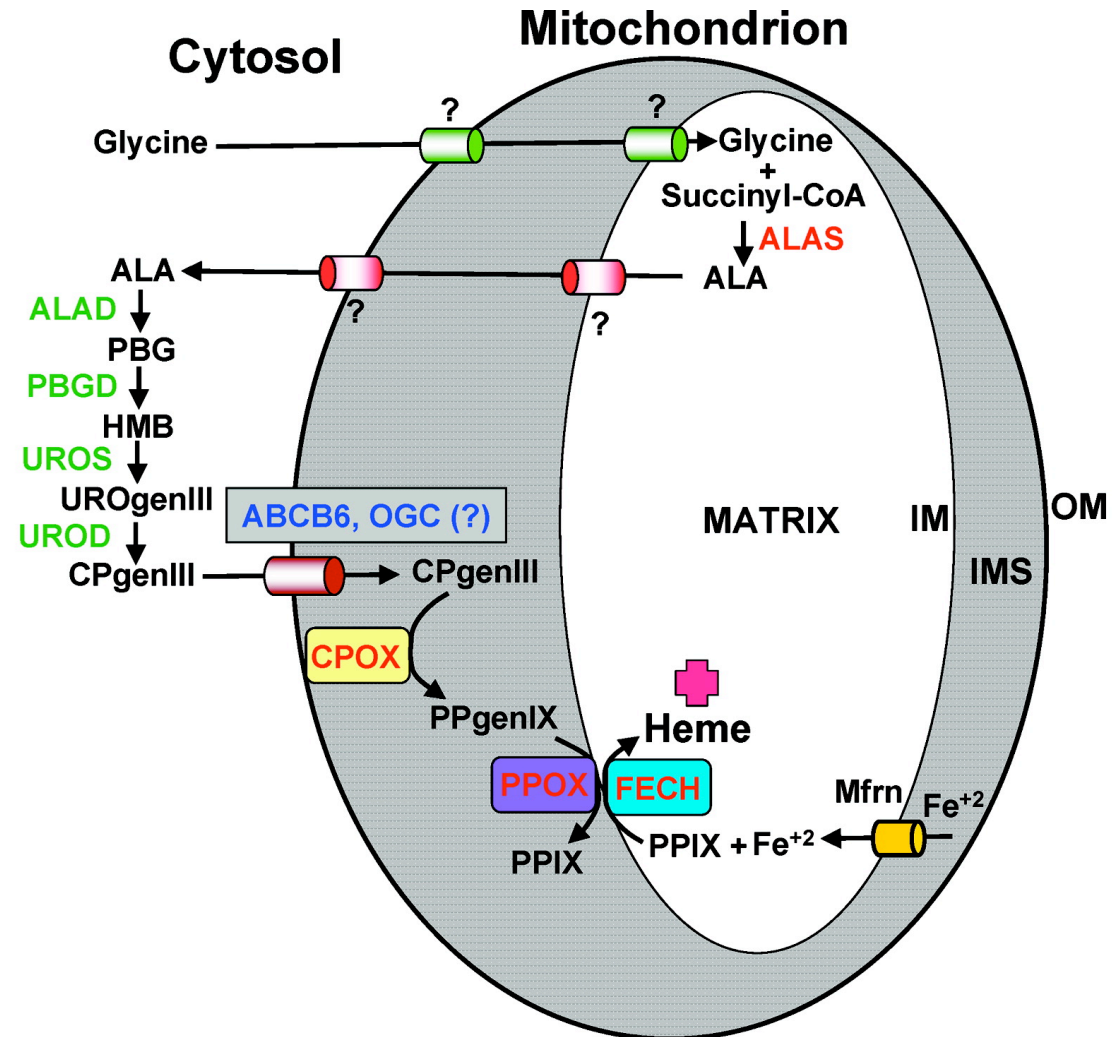
Protoporphyrin IX

A biologically relevant porphyrin

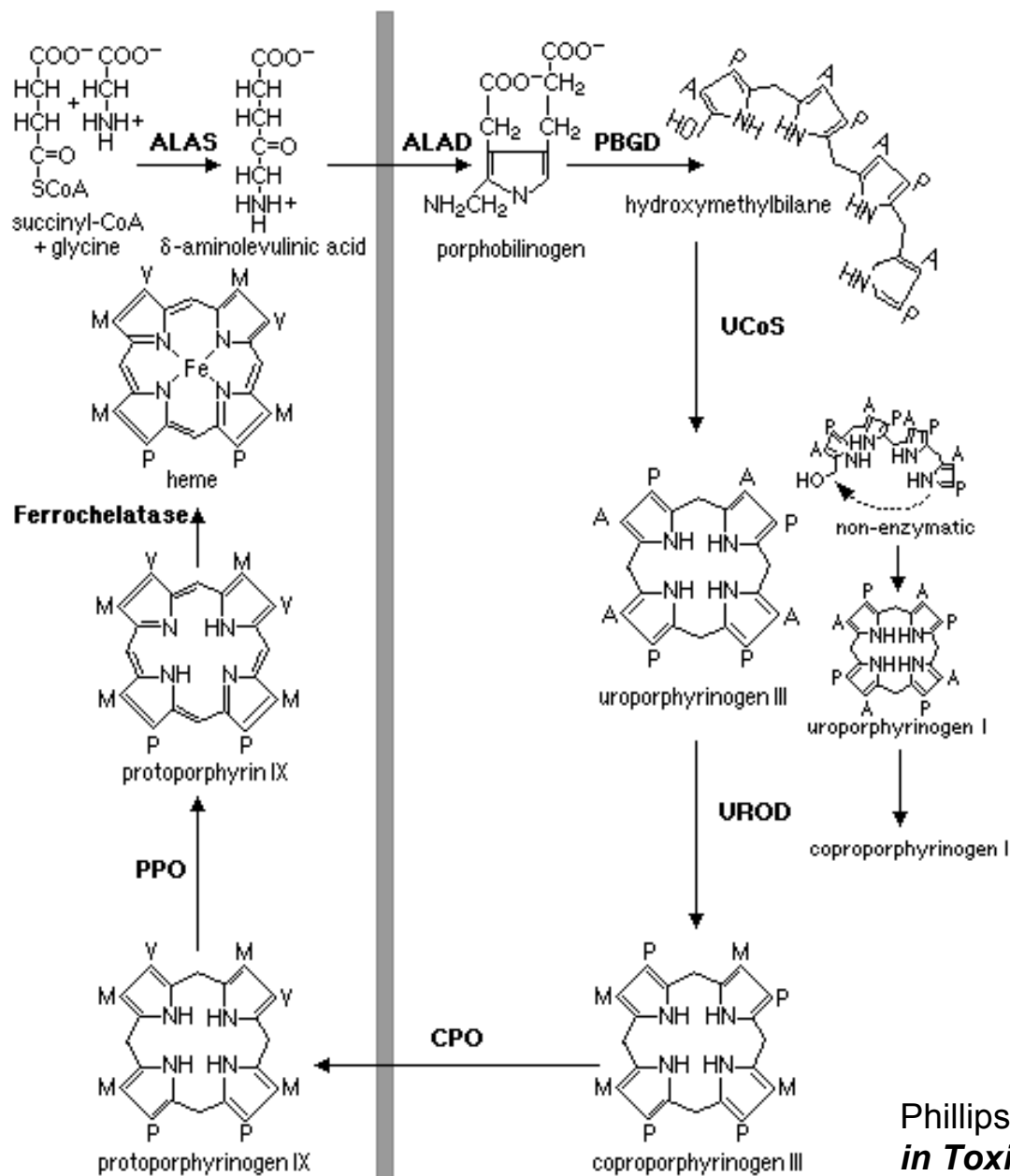


Heme biosynthesis

- Complex, multi-step process
 - Several enzymes
 - Mitochondrial
 - Cytosolic
 - Uses amino acid (glycine) and Kreb's cycle intermediate (succinyl CoA) as initial substrates
 - Terminal step involves inserting Fe^{2+} into the protoporphyrin IX skeleton to make heme *b*



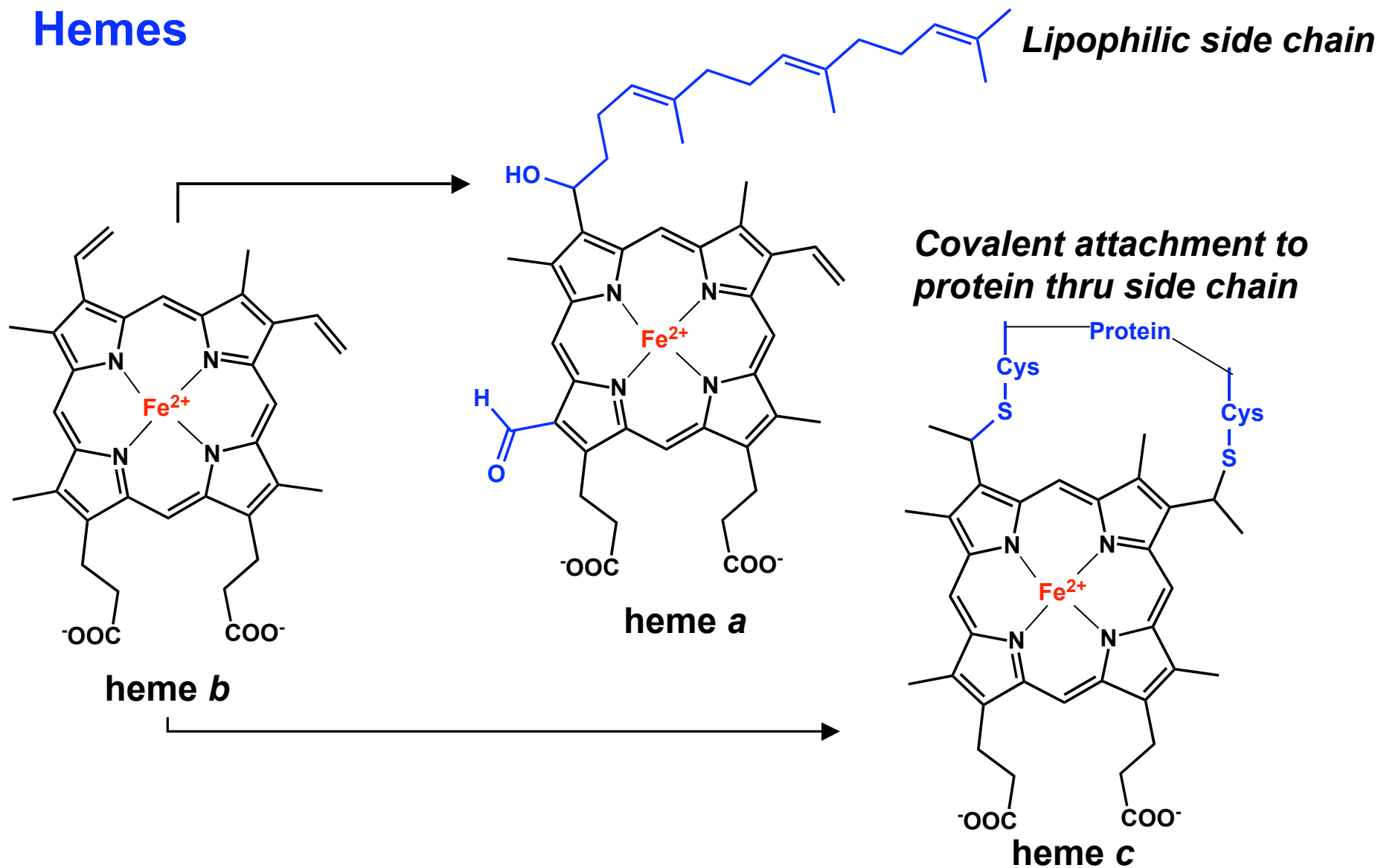
Heme biosynthesis



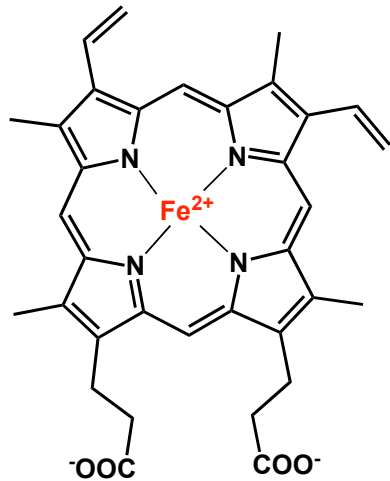
Phillips & Kushner, *Current Protocols in Toxicology*, Unit 8.1, 2001

Some biologically relevant porphyrins

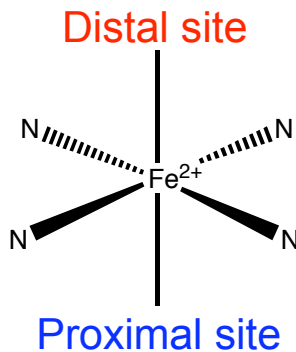
Hemes



Some heme properties correlated with function



heme b



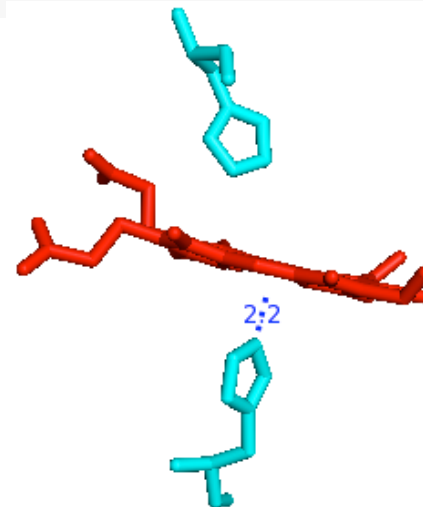
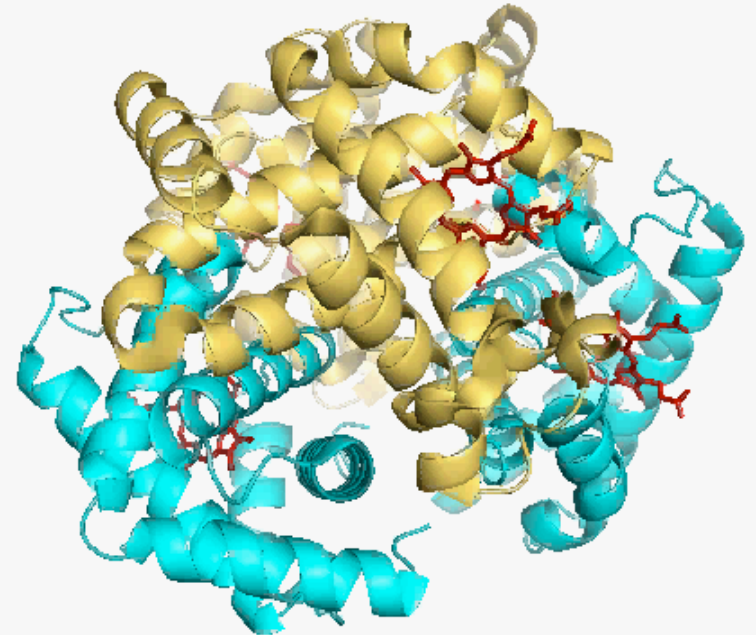
Heme coordination sites

- Resting redox state of iron (Fe²⁺ v. Fe³⁺)
- Affinity for non-protein derived ligand
 - Impacted by iron redox state
 - Some ligands bind Fe²⁺ better than Fe³⁺
- Identity of the protein-derived ligand
 - Amino acid (e.g. histidine, cysteine, methionine, tyrosine) side chain
- Shape of the heme cofactor

A survey of heme function

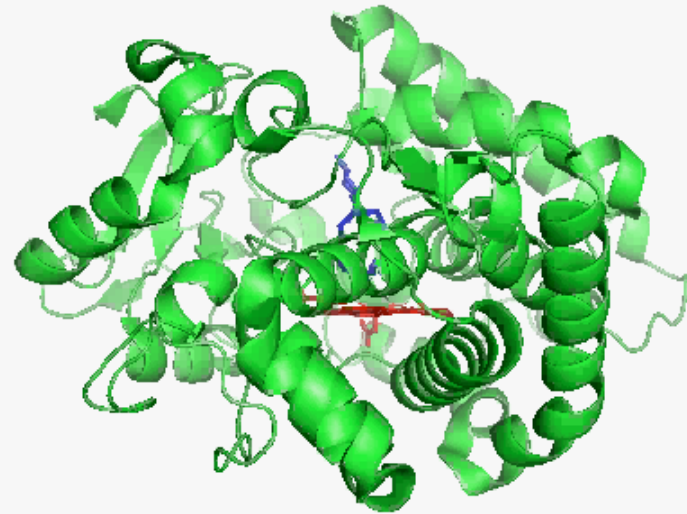
Hemoproteins and their functions

- **Function: Oxygen transport**
- **Hemoglobins**
 - *Non-protein ligand:* O_2
 - *Cofactor:* heme *b*
 - *Resting redox state:* Fe^{2+}
 - *Protein ligand to heme:* histidine
 - Tetrameric protein
 - 2 α chains
 - 2 β chains
 - Each monomeric chain binds one heme *b* molecule
 - 4 hemes/tetramer
 - Each heme can bind one O_2 atom



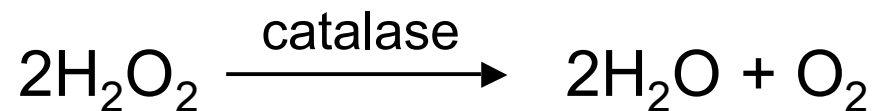
Hemoproteins and their functions

- **Enzymatic activity**
 - **Cytochrome P450s**
 - *Non-protein ligand*: O₂ (upon iron reduction to Fe²⁺ during catalytic cycle)
 - *Cofactor*: heme *b*
 - *Resting redox state*: Fe³⁺
 - *Protein ligand to heme*: cysteine
-
- **Function:**
 - Detoxify xenobiotics = foreign compounds
 - E.g. medications; environmental toxicants
 - Catalyze reactions such as: substrate oxidations



Hemoproteins and their functions

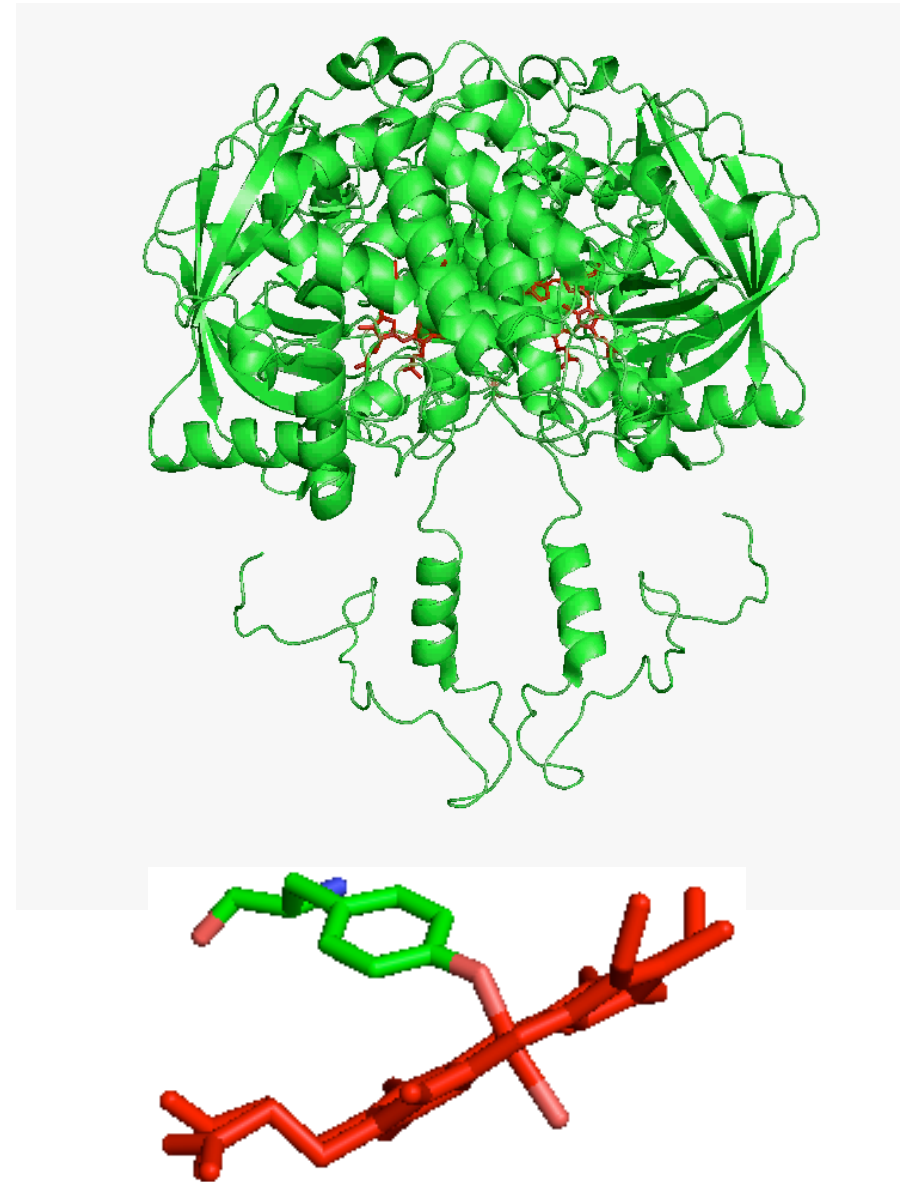
- **Enzymatic activity**
- **Catalase:**
 - *Non-protein ligand:* H_2O_2
 - *Cofactor:* heme *b*
 - *Resting redox state:* Fe^{3+}
 - *Protein ligand to heme:* tyrosine
- **Function**
 - Protects against hydrogen peroxide-induced oxidative damage
 - Breaks down hydrogen peroxide



Catalase from *H. Pylori* (PDB accession: 2IQF)

Hemoproteins and their functions

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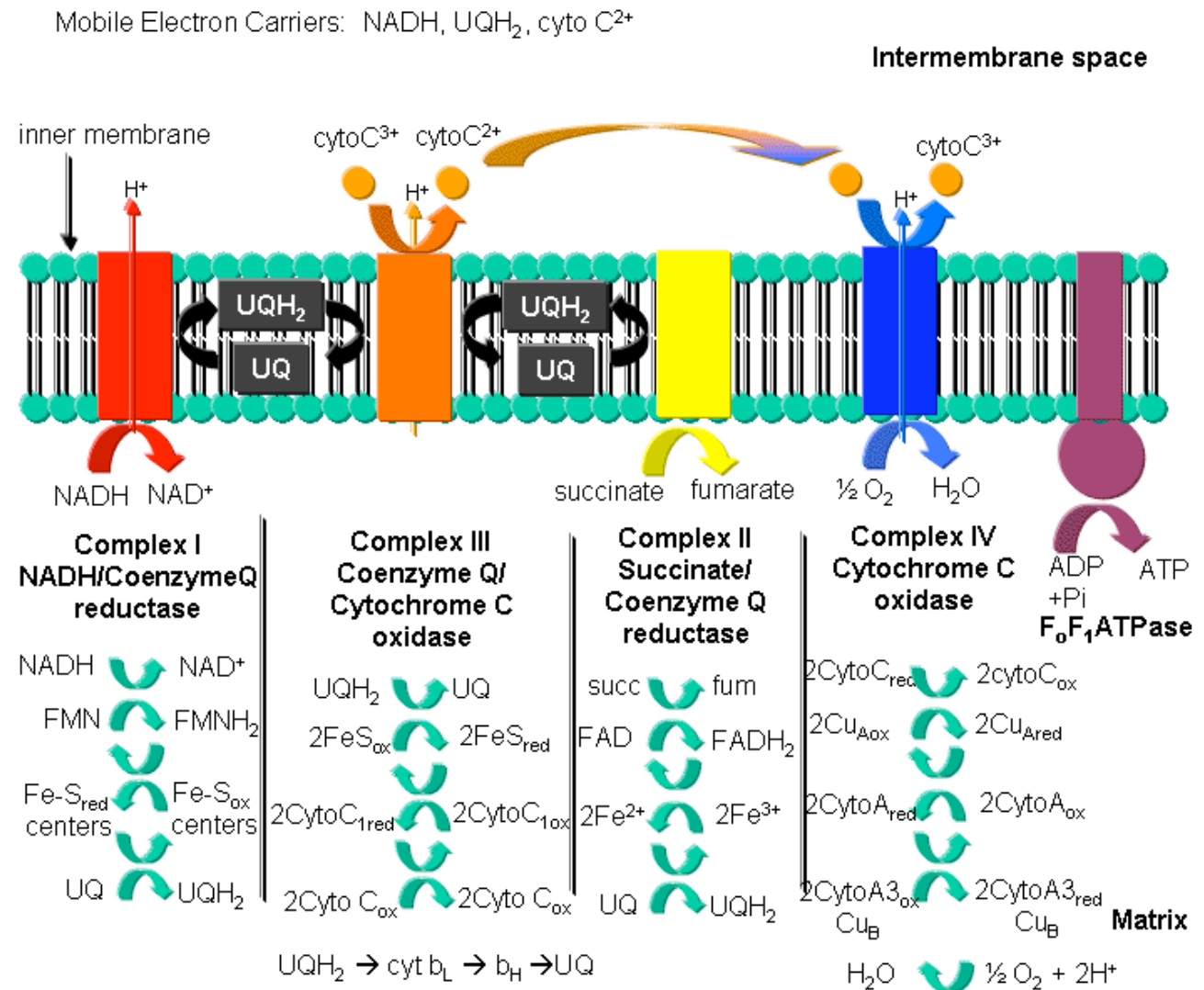


Catalase from H. Pylori (PDB accession: 2IQF)

Hemoproteins and their functions

MITOCHONDRIAL ELECTRON TRANSPORT

Electron transport chain: **cytochromes**



Hemoproteins and their functions

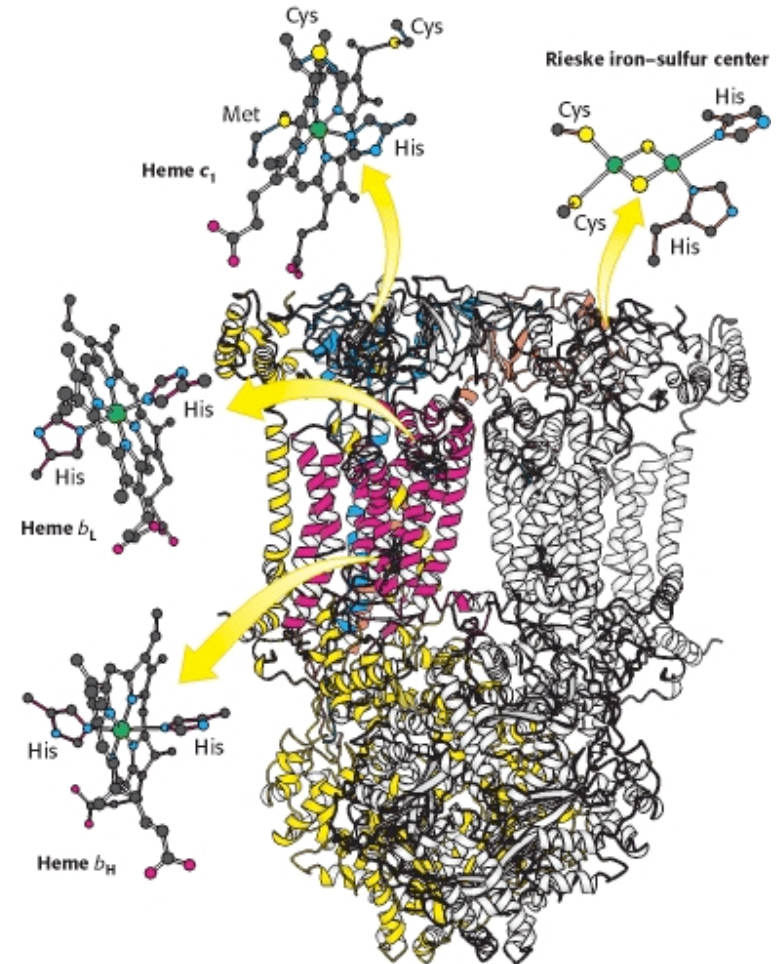
- **Electron transport chain: cytochromes**

- **Cytochrome *bc*1**

- *Non-protein ligand*: None
- *Cofactors*: 2 heme *b* + 1 heme *c*
- *Resting redox state*: Fe^{3+}
- *Protein ligand to heme*: 2 histidines

- **Function**

- Electron transfer (*not* O_2 binding) is the main function of the heme
- Bis-histidyl ligation prevents ligand binding



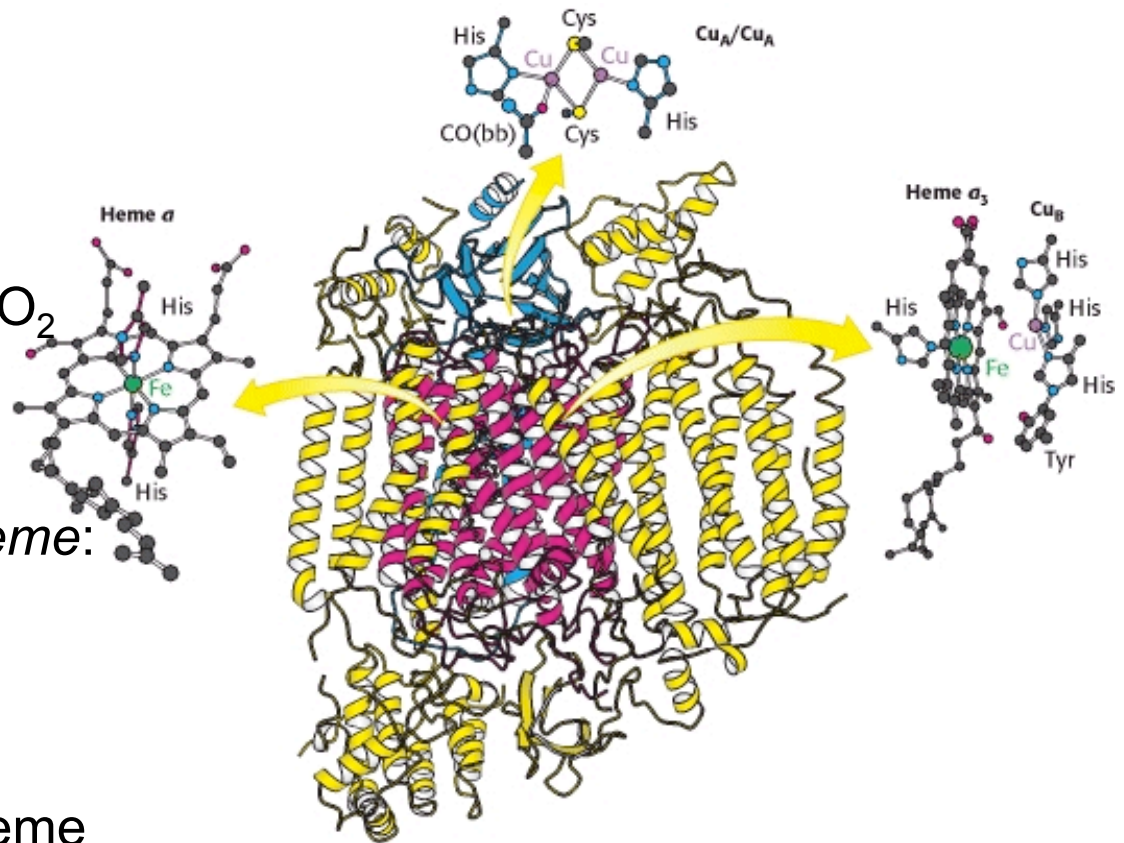
Cytochrome c oxidoreductase
(Complex III)

Hemoproteins and their functions

- **Electron transport chain: cytochromes**
- **Cytochrome c**
 - *Non-protein ligand*: None
 - *Cofactors*: 1 heme c
 - *Resting redox state*: Fe^{3+}
 - *Protein residue binding heme*: 2 histidines
- **Function:**
 - Electron transfer
 - Shuttles electrons from Complex III to Complex IV
 - Bis-histidyl ligation excludes non-protein ligand binding

Hemoproteins and their functions

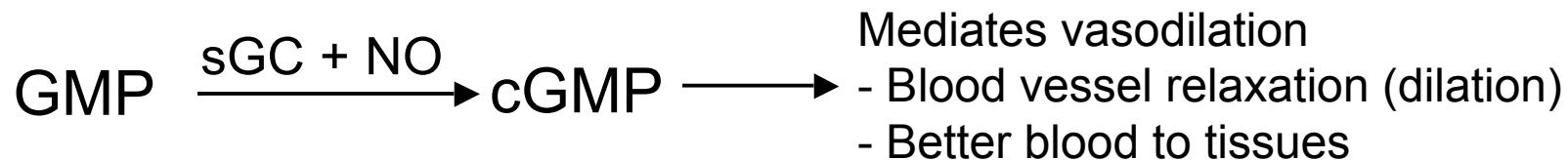
- **Electron transport chain:**
cytochromes
- **Cytochrome c oxidase**
 - *Non-protein ligand:* None/O₂
 - *Cofactors:* 2 heme a
 - *Resting redox state:* Fe³⁺
 - *Protein residue binding heme:* 1 or 2 histidines
- **Function**
 - Electron transport only (heme a – 2 histidine ligands)
 - Electron transport **AND** O₂ reduction (heme a₃ – one histidine ligand)



Cytochrome c oxidase
(Complex IV)

Hemoproteins and their functions

- **Allosteric regulation of enzymatic activity:**
- **Soluble guanylate cyclase (sGC)**
 - *Non-protein ligand*: NO (nitric oxide)
 - *Cofactors*: heme b
 - *Resting redox state*: Fe²⁺
 - *Protein residue binding heme*: 1 histidine
- **Function**
 - NO binding to heme stimulates sGC activity



Summary of heme cofactor properties

heme a cofactor

Non-protein ligand

Ligand fate

– Cytochrome c
oxidase

O₂ (heme a₃)

– Reduced to H₂O

None (heme a)

– Electron transport

heme c cofactor

– Cytochrome c

None

– Electron transport

– Cytochrome c₁

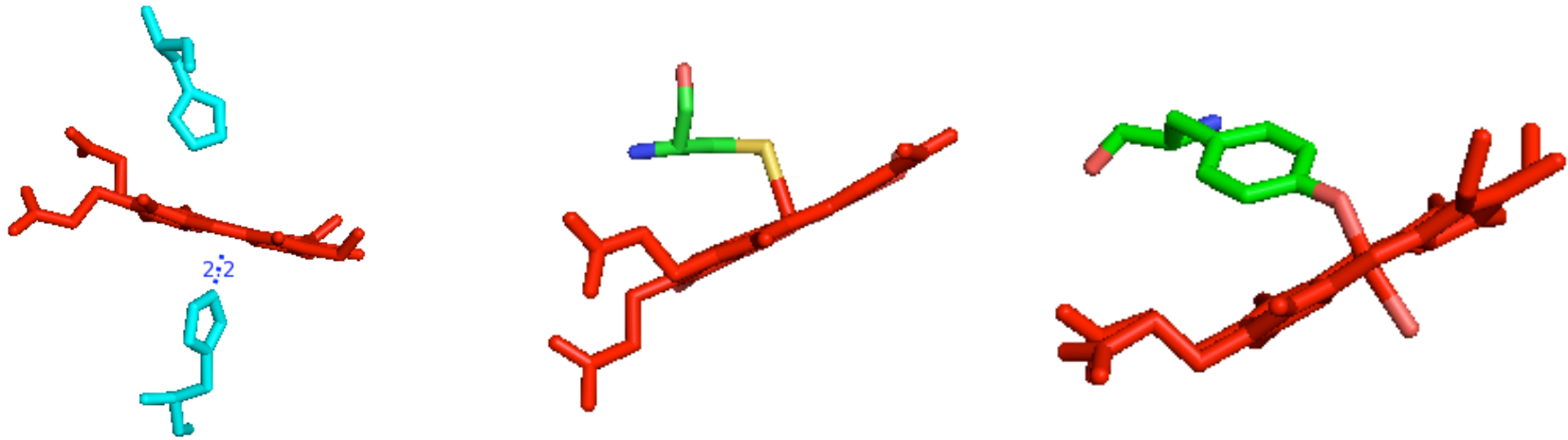
None

– Electron transport

Summary of heme cofactor properties

<i>heme b cofactor</i>	<i><u>Non-protein ligand</u></i>	<i><u>Ligand fate</u></i>
– Hemoglobin	O ₂	– Transported intact
– Cytochrome P450	O ₂	– Incorporated into product
– Catalase	H ₂ O ₂	– Degraded
– sGC	NO	– Unchanged by sGC

- Same cofactor, yet VERY different ligand binding properties
 - How might this be achieved?
 - How can the identity of the ligand binding the cofactor be tuned?
- Identical interacting ligand, yet VERY distinct outcomes possible!
 - How might this be achieved?



- Iron oxidation status
 - Fe^{2+} (O_2 , NO, CO binding favored)
 - Fe^{3+} (H_2O , H_2O_2 , CN^- (cyanide), N_3^- (azide))
- Identity of the side chains close to distal pocket
 - Block access of certain ligands
 - Stabilize bound ligand (e.g. H-bonding)
- Electron distribution in heme cofactor
 - Protein derived side chain identity
 - Heme distortion

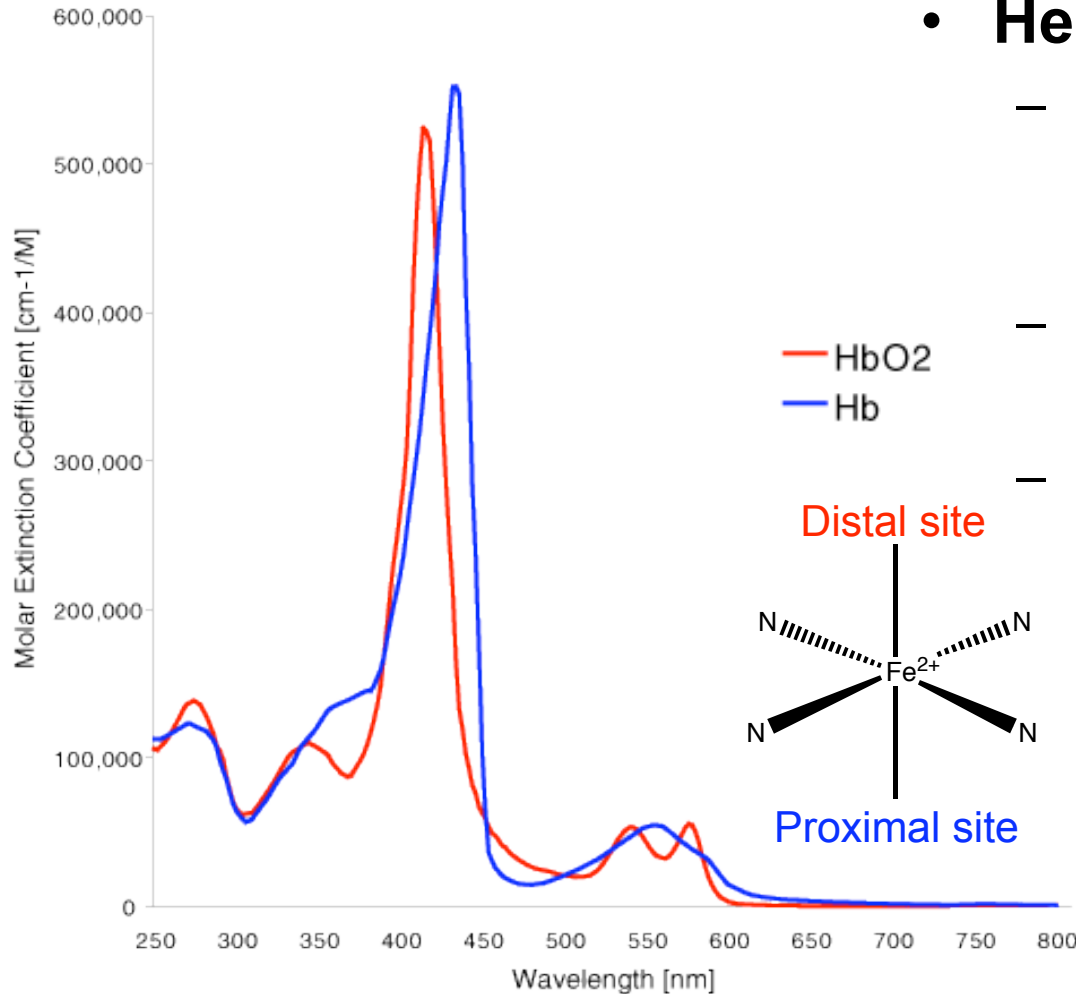
Studying hemoproteins

- Gaining insight into hemoprotein biochemistry
 - Ligand binding status
 - Oxidation state
 - Porphyrin ring distortion
- X-ray crystallographic data not always available
 - Even when available, cannot distinguish iron oxidation states

Studying hemoproteins

- Frequently used techniques:
 - Electronic absorption spectroscopy (UV-vis)
 - Iron coordination status (e.g. 5 versus 6 coordinate)
 - Iron oxidation state
 - Electron paramagnetic resonance (EPR)
 - Iron oxidation state
 - Spin state (presence of paired versus unpaired outer shell electrons)
 - Resonance Raman & Infrared spectroscopy (vibrational spectroscopy)
 - Insight into distortion of heme structure

Sample electronic absorption spectra



- **Hemoglobin**

- Maximum absorbance intensity in the 414 – 432 nm range

- “**Soret**” peak

- Soret maximum is sensitive to heme environment

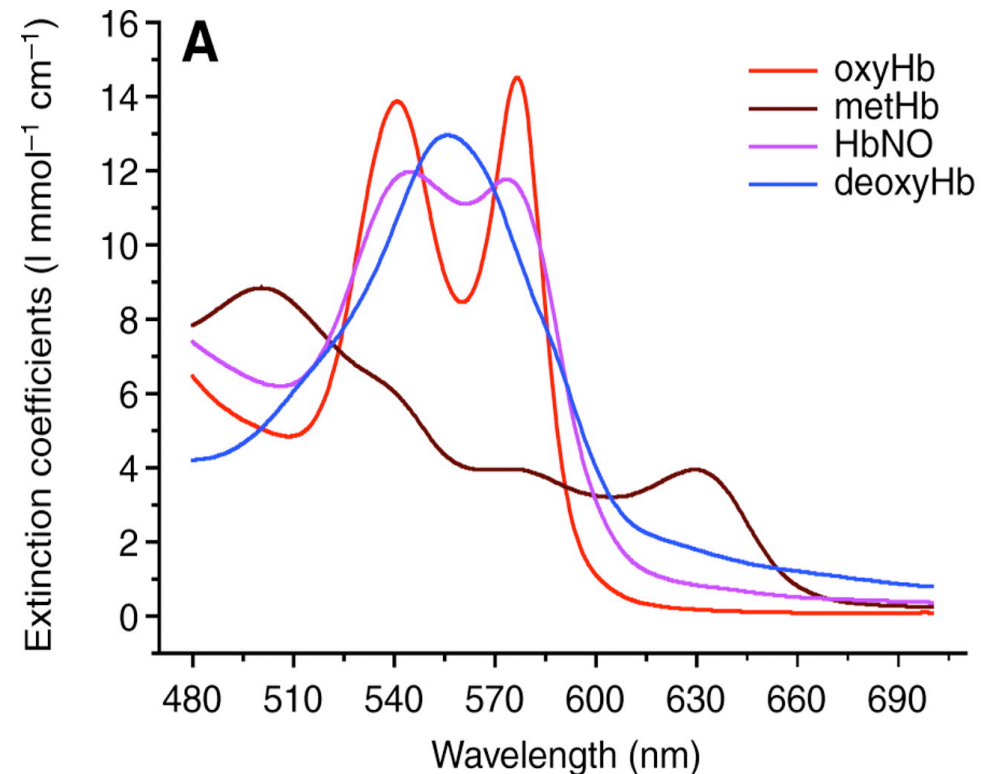
- Ligand present versus absent

- HbO₂ (6 coordinate iron) ~ 414 nm Soret

- Hb (5-coordinate) ~ 432 nm Soret

Sample electronic absorption spectra

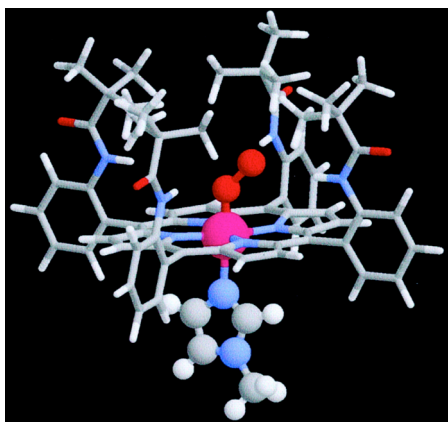
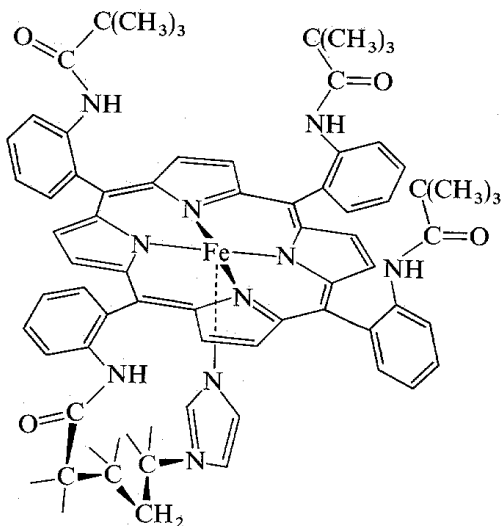
- Think of absorption spectrum as “fingerprint” for the hemoprotein state
- Absorption in this wavelength range is sensitive to the:
 - Iron oxidation state (MetHb = Fe^{3+})
 - Iron coordination state (Hb versus HbO_2)
 - Coordinated ligand (O_2 versus NO)



Modulating heme properties

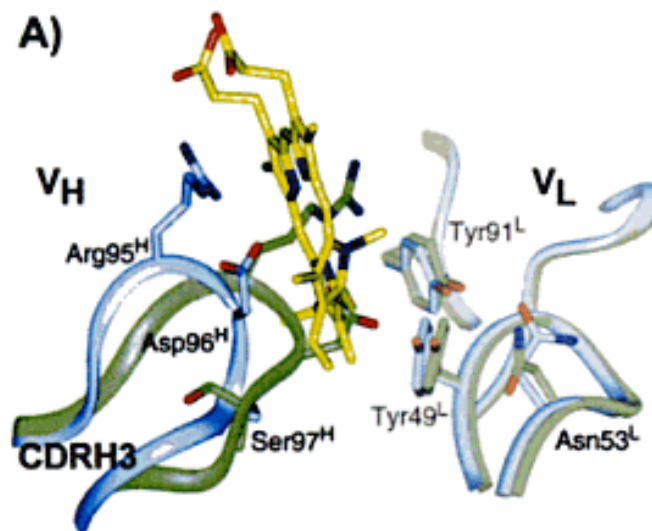
Bioorganic chemistry

Figure 22.13
A synthetic, picket fence heme complex.
[Reproduced with permission from Collman, J. P. *Acc. Chem. Res.* **1977**, *10*, 265.]



Zou S et al. PNAS 2002;99:9625-9630

Antibody



Aptamers?

Summary

- Nature uses the same basic cofactor to achieve many distinct functions:
 - Electron transfer
 - Ligand transport
 - Enzyme catalysis
 - Allosteric regulation
- These distinct functions are possible because the chemical properties of heme can be precisely tuned by its macromolecular environment
 - Nature uses several strategies to achieve the desired tuning
 - Can we selectively tune heme properties to take advantage of its rich chemistry?